Planta (1983) 158:357–364

Planta

Phototropism and polarotropism of primary chloronemata of the moss *Physcomitrella patens*: responses of the wild-type

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Abstract. Primary chloronemata growing from germinated spores of the moss *Physcomitrella patens* adopt one of two preferred polarotropic orientations depending on the wavelength and photon fluence rate of monochromatic light. Growth is mainly parallel to the electrical vector of plane polarised light in blue light and higher fluence rates of red light, and perpendicular to the electrical vector in the green and far-red regions of the spectrum and in low fluence rates of red light. The transition between the two polarotropic orientations, at wavelengths where it can be observed, usually occurs over a narrow range of fluence rates, and at this point the filaments do not grow randomly but tend to adopt in approximately equal numbers one of the preferred directions of growth. The primary chloronemata are positively phototropic in far-red light and in red light of low fluence rates, but tend to grow at right angles to the incident light in high fluence rates of red light. Simultaneous illumination with a high fluence rate of red light and a low fluence rate of far-red light causes a marked increase in the percentage of filaments growing towards the red light source at the expense of those growing at right angles to it, supporting the hypothesis that in red and far-red light, at least, the responses are controlled by the photoequilibrium of a phytochrome pool.

Key words: Bryophyta – Phototropism (moss) – Polarotropism (moss) – Moss protonemata – *Physcomitrella* – Phytochrome (moss).

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Abbreviations: E = electrical vector; P_{fr} = far-red-absorbing form of phytochrome; P_r = red-absorbing form of phytochrome; P_{tot} = total phytochrome ($P_r + P_{fr}$)

Introduction

Phototropism is observed in a variety of plant species in both multicellular organs and filamentous structures. In a number of cases the responses have been characterised in some detail and the photoreceptors involved have been identified (Dennison 1979). For example, a blue-light receptor controls the phototropic responses of the oat coleoptile (Thimann and Curry 1960) and the *Phycomyces* sporangiophore (Delbrück and Shropshire 1960), whereas in protonemata of the fern *Adiantum capillus-veneris* (Kadota et al. 1982) and the moss *Physcomitrium turbinatum* (Nebel 1968) phytochrome is the effective photoreceptor. Both photoreceptor systems appear to be involved in the tropic responses of *Dryopteris felix-mas* protonemata (Etzold 1965; Steiner 1969a). In the related polarotropic responses, growth is aligned with respect to the orientation of the electrical vector (E) of plane polarised light, providing strong evidence for an ordered arrangement of the photoreceptors concerned, in a stable structure near the periphery of the cell. A similar conclusion was drawn by Haupt et al. (1969) for the phytochrome molecules controlling chloroplast orientation in the alga *Mougeotia*. Most recently, Kadota et al. (1982) have reported that the red- and far-red-absorbing forms of phytochrome (P_r and P_{fr} respectively) involved in the tropic responses of *Adiantum* protonemata are in different dichroic orientations at the cell flank.

The moss *Physcomitrella patens* is particularly well suited to studies of photo- and polarotropism. Each of the three types of protonemal filament (the caulonemata and the primary and secondary chloronemata) and the multicellular gametophores exhibit a different and characteristic set of re-

sponses, some of which are controlled by phytochrome (Cove et al. 1978). However, perhaps the greatest advantage of using *Physcomitrella* is that it is possible to combine physiological investigations with a genetic approach through the isolation and characterisation of mutant strains altered in their tropic responses. In the present paper we describe the photo- and polarotropism of primary chloronemata which grow following germination of wild-type *Physcomitrella* spores.

Material and methods

Strain and media. The wild-type *Physcomitrella patens* strain used here was derived from a single spore. Ashton and Cove (1977) give details of the origin of the wild-type and describe techniques for routine sterile culture and the preparation of spore suspensions. The minimal medium described by Ashton and Cove (1977) was used, except that 5 mM $Ca(NO_3)_2 \cdot 4\,H_2O$ and 5 mM ammonium tartrate were added as supplements after autoclaving, and the medium was adjusted to pH 6.5 before the addition of 1.2% (w/v) agar (Difco Bacto Agar). Sucrose (0.5%, w/v) was added to permit growth at low photon fluence rates, and 1.8 μM *p*-aminobenzoic acid and 0.3 μM thiamine HCl were included so that the same medium could be used for strains having an auxotrophic requirement for these vitamins.

Induction of germination. The medium was poured into 5-cm-diameter, shallow, plastic Petri dishes. Two drops of a spore suspension containing approximately $2.5 \cdot 10^3$ viable spores ml^{-1} were placed in the centre of the plates using a pasteur pipette. Since the light requirements for spore germination and the growth of primary chloronemata are different, germination was induced before the spores were transferred to the experimental chambers. This was done by illuminating the spores from above with 15 W m^{-2} white light provided by fluorescent tubes (40 W, Reflectalite; Philips, Croydon, UK) for approx. 44 h at 25° C (Cove et al. 1978). At the end of this period, primary chloronemata were just emerging from at least 50% of the spores.

Illumination for photo- and polarotropism. Germinated spores were illuminated at 25° C either unilaterally with non-polarised monochromatic light, or from above with plane-polarised monochromatic light. Light was provided by Aldis Tutor 2 projectors (Rank Audio Visual Ltd., Brentford, UK) with thyristor dimmer controls and 250-W tungsten-halogen bulbs (type M36; Thorn Lighting, Birmingham, UK). The light passed through filters (DAL type, narrow band, double interference filters, half

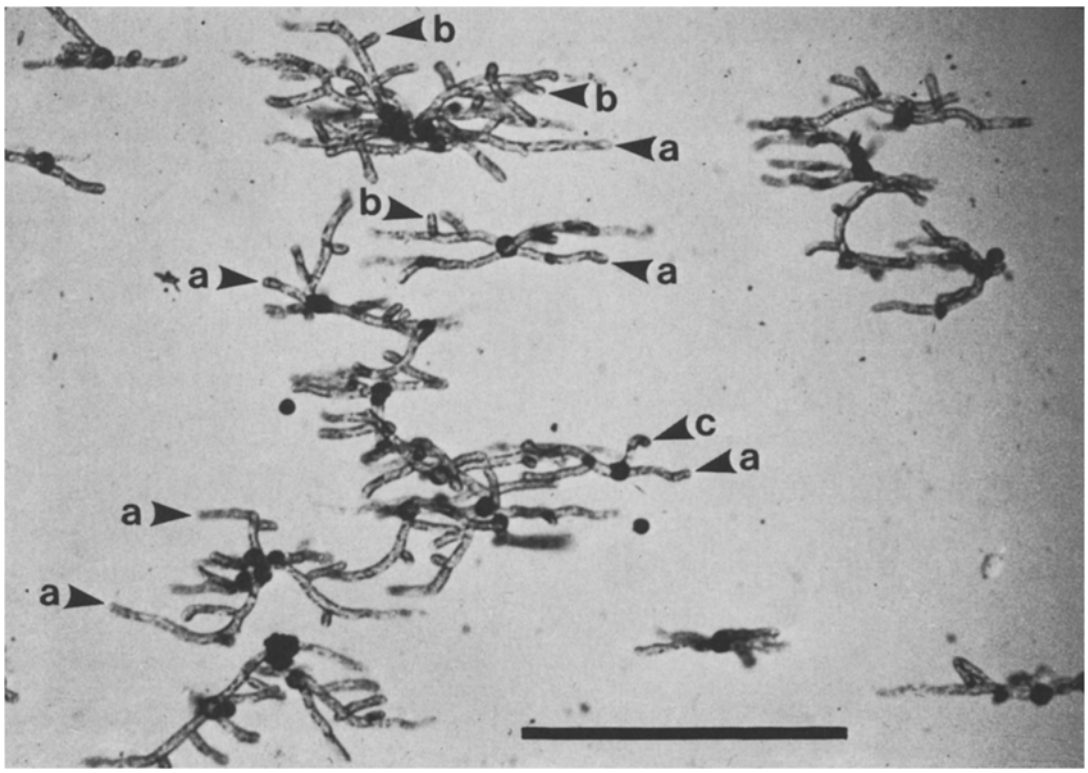

Fig. 1. Primary chloronemata of *Physcomitrella* which have grown from spores in plane polarised monochromatic light (665 nm, 8.8 μmol quanta $m^{-2}s^{-1}$). The orientation of the electrical vector (*E*) is parallel to the bar. Wild-type spores were inoculated onto solid supplemented minimal medium in a Petri dish and were first illuminated for 44 h with white light (15 W m^{-2}) to induce germination, before being transferred to polarised monochromatic light for a further 72 h. Illumination throughout was from above and chloronemal filaments grew at right-angles to the light direction. Data on the orientation with respect to *E* of the long axis of the apices of filaments were collected from this type of material. Only axial filaments were scored (*a*); side branches (*b*) and filaments with curved apices (*c*) were not included in the analysis. Bar = 1 mm

band width 16–20 nm, Schott, Mainz, FRG; glass polarising filters, H.S.B. Meakin Ltd., London, UK) into otherwise light-proof experimental chambers. Because plastic Petri dishes depolarise polarised light, for experiments where illumination was with polarised light, Petri dish lids were replaced by 5-cm squares of thin plate glass. The plastic Petri dishes do not polarise light and so do not interfere with experiments using unpolarised-light treatments. Light was measured with a radiometer (type J16 with a J6512 probe, Tektronix UK Ltd., London, UK).

Analysis of responses. After about 72 h in monochromatic light, the point of incidence of unidirectional light or the plane of the electrical vector of polarised light was marked on the Petri dishes and, using a microscope, the direction of growth of the primary chloronemal tip cells was scored with respect to these marks. The tip cells were aligned with parallel lines on a graticule in an eyepiece which could be rotated against a circular scale marked in 10° units. Care was taken to score only the most recent linear direction of growth of the axial filaments. As shown in Fig. 1, filaments growing in an arc were not scored, and neither were side branches or filaments that were just emerging from the spore. Normally 40–120 filaments were scored per plate.

Results

Growth of the primary chloronemata. The primary chloronemata do not grow in darkness even when an exogenous carbon source (sucrose) is present in the medium. Growth in the presence of sucrose ceases in monochromatic 665-nm light at photon fluence rates below approx. 0.15 μmol $m^{-2}s^{-1}$, and in 442-nm light below approx. 0.5 μmol $m^{-2}s^{-1}$. At other wavelengths, sufficient growth occurs to score tropic responses at fluence rates down to about 1 μmol quanta $m^{-2}s^{-1}$. Primary chloronemata grown in monochromatic light of wavelengths between 417 and 687 nm branch frequently, have cross walls predominantly at right-angles to the long axis of the filaments and contain numerous well-developed chloroplasts. In contrast, in monochromatic far-red light (715–760 nm) the primary chloronemata have few, chlorophyll-deficient plastids, the filaments seldom branch, and become extremely elongated.

Polarotropism. The polarotropic response of primary chloronemata in 665-nm light of 8.8 μmol $m^{-2}s^{-1}$ is shown in Fig. 1. When only the tip cells of axial filaments are considered, it is evident that the majority are aligned essentially parallel to *E*. Although many filaments branch and alter their direction of growth, the response of the population as a whole does not change significantly between 24 and 120 h after transfer to monochromatic red light (data not shown). This is also observed at other wavelengths over a wide range of fluence rates.

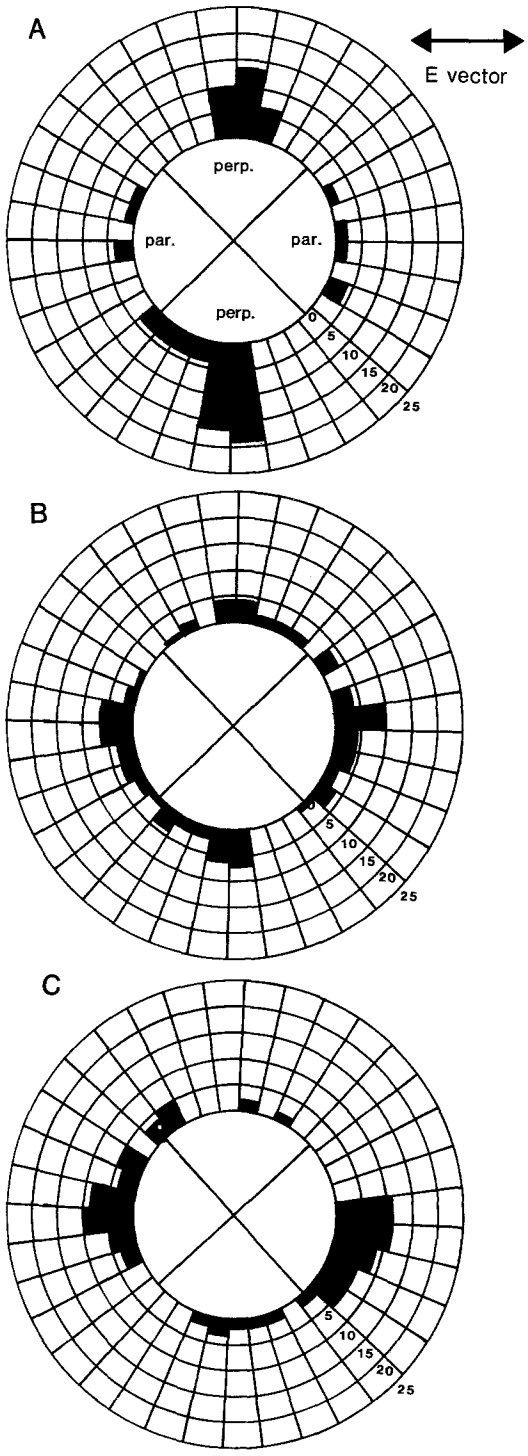

Fig. 2A–C. Histograms showing the percentage distribution of orientations adopted by primary chloronemal filaments of *Physcomitrella* growing in plane polarised monochromatic light (665 nm; **A** 0.8 μmol quanta $m^{-2}s^{-1}$; **B** 1.7 μmol quanta $m^{-2}s^{-1}$; **C** 10 μmol quanta $m^{-2}s^{-1}$). Each concentric circle represents a 5% increment as indicated by the numerical scale. The orientation of the electrical vector (*E*) is shown. The experimental conditions and method of scoring are described in the text and the legend to Fig. 1. The quadrants which comprise filaments growing parallel or perpendicular to *E* (two quadrants for each) used in later analyses, are indicated in **A**

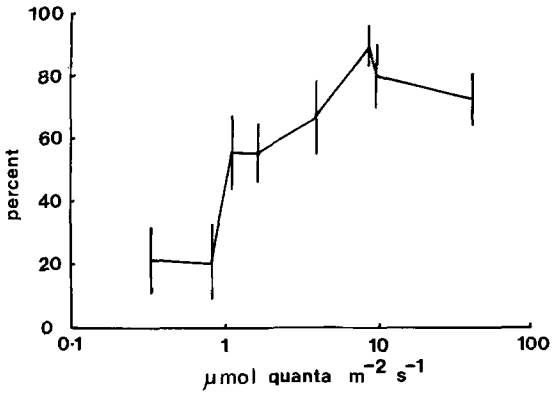

Fig. 3. The effect of intensity of plane polarised monochromatic light (665 nm) on the orientation of primary chloronemal filaments of *Physcomitrella*. The percentage of filaments oriented parallel to the electrical vector is plotted against photon fluence rate. The bars give the 95% confidence limits of each point. The experimental conditions and method of scoring are described in the text and the legends to Figs. 1 and 2.

The directions of growth of the primary chloronemata were recorded on circular histograms. The results presented in Fig. 2 show that in polarised 665-nm light, growth is predominantly parallel to E in relatively high fluence rates, but mainly perpendicular to E below approx. 1 μmol $m^{-2}s^{-1}$. At intermediate fluence rates, the filaments tend to adopt one of the two preferred polarotropic orientations in approximately equal numbers, other directions of growth being less favoured. In order to describe quantitatively the response at any given wavelength, the circular histograms obtained for each fluence rate were divided into quadrants (see Fig. 2) and the percentage of filaments growing in the two quadrants parallel to E was calculated. The results obtained for 665-nm light (Fig. 3) show the transition from the 'low-light' perpendicular polarotropic response to the 'high-light' parallel response.

Since we observe the growth response of a population of filaments growing in continuous illumination, it is not possible to establish a dose-response relationship, so an action spectrum for the polarotropic response cannot yet be determined. However, in Fig. 4, the percentage of filaments growing parallel to E at various wavelengths and photon fluence rates is plotted, summarising our data. Observations at lower fluence rates were limited by low rates of chloronemal growth. The primary chloronemata tend to grow parallel to E in blue light and in higher fluence rates of red light, and perpendicular to E in green and far-red light and in lower fluence rates of red light. The tendency of filaments not to favour intermediate directions of growth is seen in the circular histograms at all wavelengths (data not shown). At all but one of the wavelengths where a transition from perpendicular to parallel polarotropism is observed, the crossover occurs over the narrow range of approx. 0.4 μmol $m^{-2}s^{-1}$. The exception is at 637 nm, where the transition occurs between 0.6 and 6.0 μmol $m^{-2}s^{-1}$.

Phototropism. The phototropic response of primary chloronemata in unidirectional light is also dependent on wavelength and photon fluence rate. In 665-nm light (Fig. 5) most filaments are positively phototropic at fluence rates below approx. 5 μmol $m^{-2}s^{-1}$, but tend to grow at right-angles to the incident light at high fluence rates. At intermediate fluence rates, growth is spread between

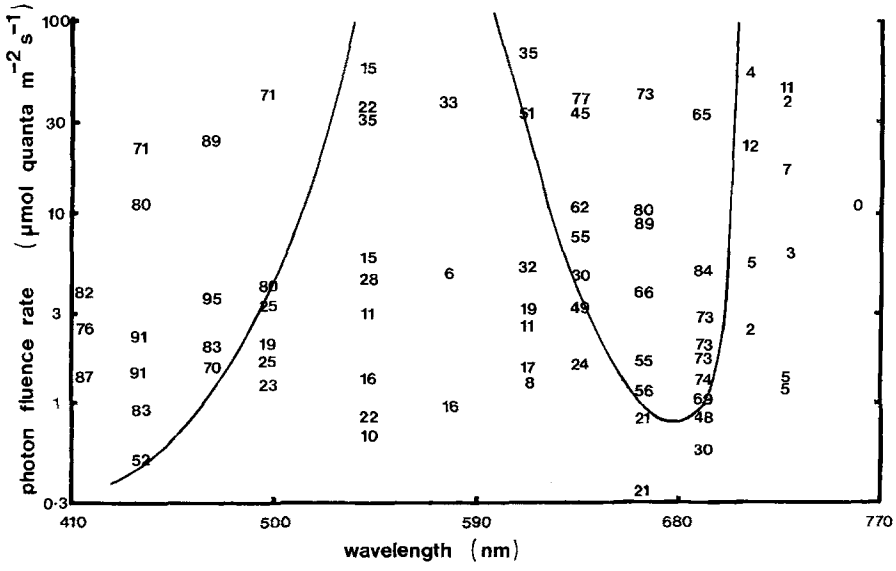

Fig. 4. Percentage of *Physcomitrella* filaments growing parallel to the electrical vector of polarised light at various wavelengths and photon fluence rates. The experimental conditions and methods of scoring are described in the text and the legends to Figs. 1 and 2. The line attempts to represent the 50% 'contour'

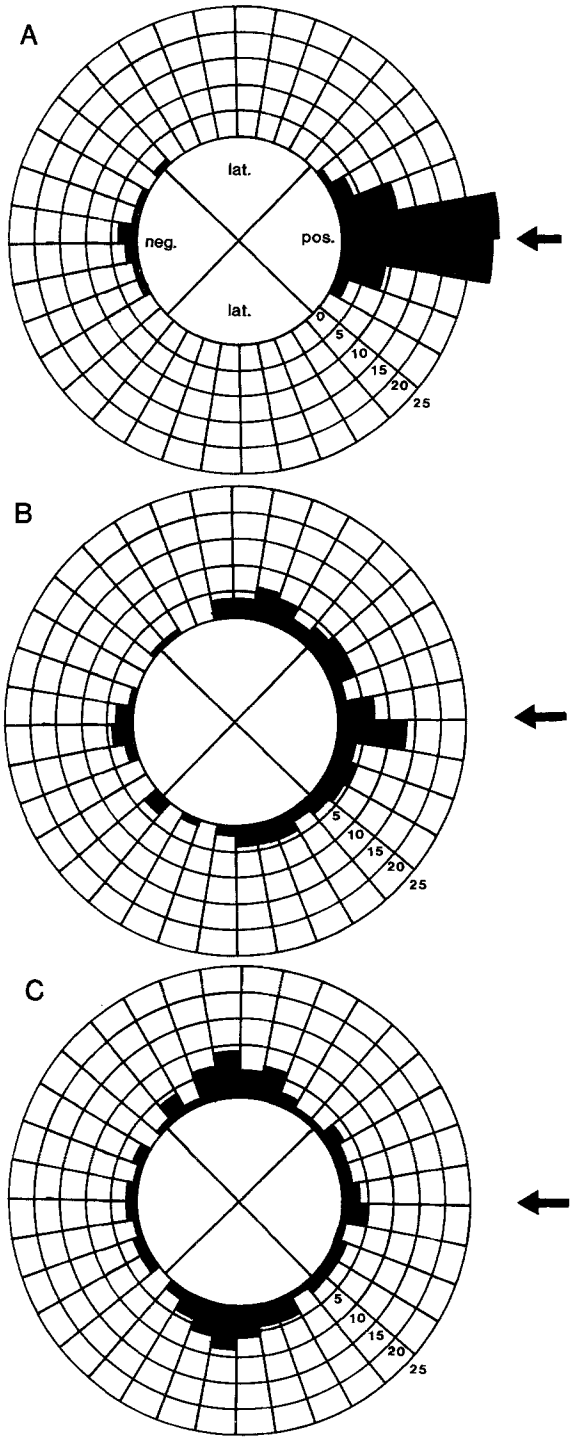

Fig. 5A–C. Histograms showing the percentage distribution of orientations adopted by primary chloronemal filaments of *Physcomitrella* which have grown from spores in unidirectional monochromatic light (665 nm; **A** 0.5 μmol quanta $m^{-2}s^{-1}$; **B** 5.5 μmol quanta $m^{-2}s^{-1}$; **C** 39 μmol quanta $m^{-2}s^{-1}$). The experimental procedure was essentially similar to that described in the legend of Fig. 1 except that an unpolarised beam of monochromatic light was shone through the edge of the Petri dish. The direction of illumination was from the left as indicated by the arrows. The quadrants which comprise filaments showing positive and negative phototropism (90° quadrant for each) or growing laterally (two 90° quadrants), used in later analyses, are indicated in **A**

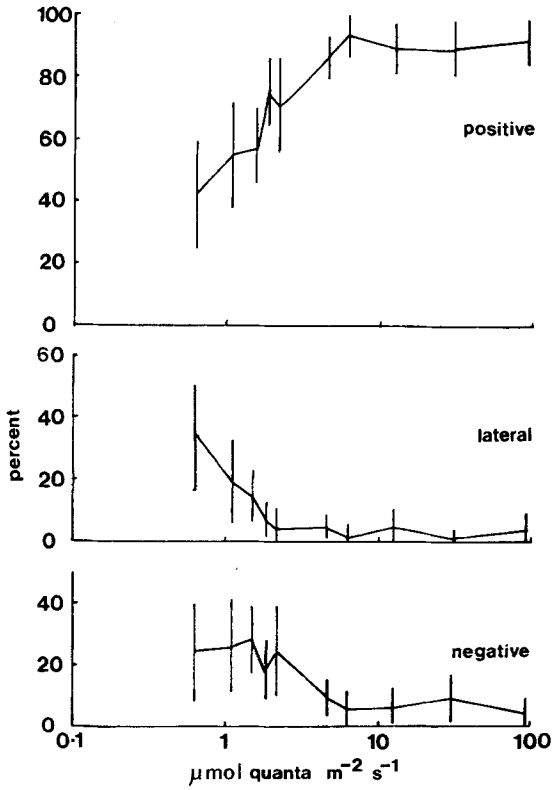

Fig. 6. The effect of intensity of unidirectional monochromatic light (665 nm) on the orientation of primary chloronemal filaments of *Physcomitrella*. The percentage of filaments growing towards (upper graph), lateral to (middle graph) and away from (lower graph) the light source is plotted against photon fluence rate. The bars give the 95% confidence limits of each point. The experimental conditions and methods of scoring are described in the text and legend to Fig. 5

these two extremes. The circular histograms were divided into quadrants and the percentage of filaments growing either towards, away from, or at right-angles to the light was calculated. As shown in Fig. 6, the percentage of negatively phototropic filaments is always low in 665-nm light. In 730-nm light the filaments are positively phototropic over a wide range of fluence rates and show very little tendency towards lateral growth (Fig. 7).

Simultaneous illumination with red and far-red light. In order to test the involvement of phytochrome in these responses, phototropism of the primary chloronemata was examined under simultaneous illumination with monochromatic red and far-red light. In one type of experiment, the germinated spores were illuminated unilaterally with a relatively high fluence rate of 665-nm light and from exactly the opposite direction with a low fluence rate of 730-nm light. The responses of the primary chloronemata under these conditions are shown in Table 1A. As described above, the red and far-

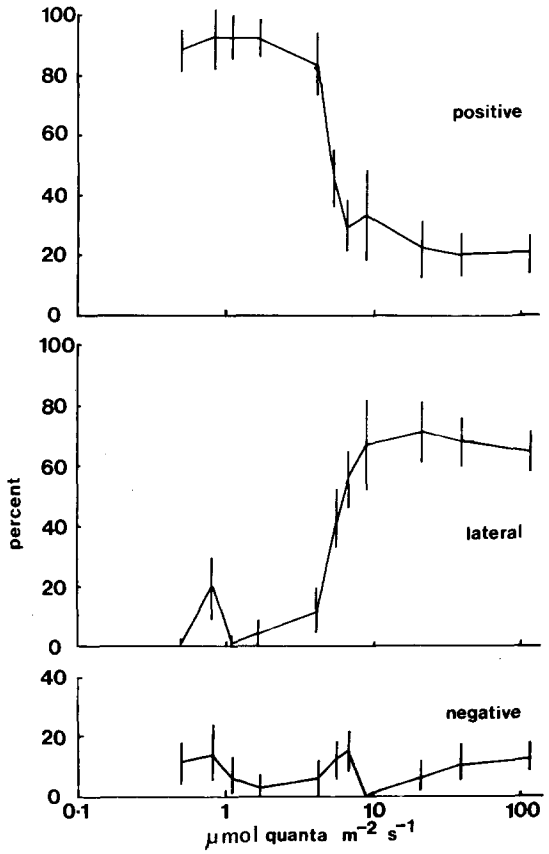

Fig. 7. The effect of intensity of unidirectional monochromatic light (730 nm) on the orientation of primary chloronemata of *Physcomitrella*. Experimental details are as for Fig. 6

red treatments alone result in strong lateral or positive phototropic responses respectively. Simultaneous illumination causes a marked decrease in the percentage of filaments growing at right-angles to the light source, and a large increase in the percentage growing towards the red light. In a second type of experiment, similar conditions were employed except that the non-polarised far-red light was given from above. This treatment alone causes the filaments to grow out of the agar where they become desiccated and wither, and thus no tropic responses can be scored. However, simultaneous illumination under these conditions again increases the percentage of filaments growing towards the red light at the expense of those growing laterally (Table 1 B).

Table 1 A, B. The effects of simultaneous illumination with non-polarised monochromatic red (665 nm) and far-red (730 nm) light on the growth of chloronemal filaments of *Physcomitrella*. The experimental procedures were essentially similar to those described in the legend to Fig. 5 except that in **A** the red and far-red illuminations were in exactly opposite directions in the horizontal plane through the edge of the Petri dish whereas in **B** the red light was directed horizontally through the edge of the Petri dish as usual while the far-red light was shone from above. The data are representative of a number of experiments which gave similar results. When illuminated with far-red light from above alone, filaments grow out of the agar towards the light source where they become desiccated and wither. No filaments grow in the plane of the agar surface

A Red and far-red light through edge of Petri dish from opposing directions

Photon fluence rate (μmol quanta $m^{-2}s^{-1}$)		Chloronemal filaments growing [%]			Number of filaments scored
Red	Far-red	Towards red and – or away from far-red	Lateral to red and – or far-red	Towards far-red and – or away from red	
16.6	0	22.2±4.6	71.0±5.0	6.8±2.8	81
16.6	1.8	59.0±6.3	4.9±2.8	36.1±6.1	61
0	1.8	18.2±4.7	6.8±3.1	75.0±5.3	66
16.6	2.1	64.8±4.7	13.8±3.4	21.4±4.0	105
0	2.1	25.0±7.4	4.4±3.5	70.6±7.8	34

B Red light through edge of Petri dish, far-red light from above

Photon fluence rate (μmol quanta $m^{-2}s^{-1}$		Chloronemal filaments growing [%]			Number of filaments scored
Red	Far-red	Towards red	Lateral to red	Away from red	
111	0	21.5±3.0	65.7±3.4	12.8±2.4	191
111	2.1	41.1±3.5	41.9±3.5	17.0±2.7	197
0	2.1	No response can be scored (see legend)			

Discussion

The primary chloronemata of *Physcomitrella* exhibit tropic responses over the wide range of wavelengths and photon fluence rates that support growth. Random growth is not observed even at the transition between one preferred tropic orientation and another. Such close coupling between growth per se and the ability to grow directionally is not seen in all species. For example, in the liverwort *Sphaerocarpos* the primary chloronemata are polarotropic in blue light, but grow randomly in polarised light of wavelengths greater than 550 nm (Steiner 1969b). Light clearly has additional major influences on the growth and development of *Physcomitrella* primary chloronemata, but the tropic responses appear to be largely independent of, for

example, filament growth rate, time after emergence from the spore, the degree of branching, and the extent of plastid development. Thus similar tropic responses are observed in green and far-red light even though the morphology of the filaments is quite different. This demonstrates that various aspects of chloronemal development are under different photocontrols.

The *Physcomitrella* primary chloronemata adopt one of two preferred polarotropic orientations, either parallel to or perpendicular to *E*, depending on the wavelength and fluence rate. This is in contrast to species such as *Dryopteris* (Etzold 1965), *Sphaerocarpos* (Steiner 1969b) and *Adiantum* (Wada et al. 1981), where only a response perpendicular to *E* has been reported. *Physcomitrella* also shows two phototropic orientations, growing either towards or at right-angles to the incident light, but the transition from one to the other is less sharp than for the polarotropic responses, and occurs at a slightly higher photon fluence rate. A likely explanation for this is that in our experiments non-polarised, unidirectional light has to pass through up to 2 cm of agar before it reaches the spores and scattering undoubtedly occurs, whereas polarised light given from above encounters no such barrier.

The present results differ in a number of respects from those reported by Nebel (1968) for chloronemata of the moss, *Physcomitrium turbinatum*. Nebel found that blue and far-red light alone were ineffective in inducing phototropism in *Physcomitrium*, although far-red light became very effective when red light was given simultaneously. In contrast, tropic responses in blue and far-red light can readily be observed in *Physcomitrella*. A further point of difference is that lateral phototropism was not observed in *Physcomitrium*. It should be noted, however, that the null-balance method used by Nebel to compare the effectiveness of two light sources, differing in their angle of incidence on the protonemata by 90°, cannot easily distinguish between a minimal positive phototropic response and lateral phototropism.

In attempting to identify the photoreceptors concerned with the photo- and polarotropic responses of *Physcomitrella* primary chloronemata, it is necessary to account for action over a wide range of wavelengths. The fact that different responses are observed in red and far-red light, and that phytochrome controls phototropism of *Physcomitrella* caulonemata and gametophores (Cove et al. 1978), led us to investigate the involvement of this photoreceptor. We have started to test the hypothesis that the parallel polarotropic and lateral phototropic responses occur when a relatively high ratio of P_{fr} to total phytochrome (P_{tot}) is present in the cells, and that the perpendicular polarotropic and positive phototropic responses occur when the ratio is below a certain critical value. Simultaneous illumination with a high fluence rate of red light and a low fluence rate of far-red light should decrease the P_{fr}/P_{tot} ratio sufficiently to induce a positive rather than a lateral phototropic response. This is the interpretation of the experiments described in Table 1; superimposition of the responses observed under red and far-red light alone cannot account for the results obtained under simultaneous illumination, whether far-red is applied laterally or from above.

In order to account for the tropism observed in red light by the above hypothesis, the P_{fr}/P_{tot} ratio must drop below the proposed critical value at low photon fluence rates. It is therefore of interest that Heim and Schäfer (1982) have recently described the fluence-rate-dependence of P_{fr}/P_{tot} under continuous illumination with red light in seedlings of *Sinapis alba,* low P_{fr}/P_{tot} ratios being observed at low fluence rates. Whether the tropic responses of the primary chloronemata at all wavelengths can be explained according to the P_{fr}/P_{tot} ratio of the relevant phytochrome pool is a matter for conjecture. It is possible that sufficient P_{fr} is generated in blue light to account for the parallel polarotropic response or, alternatively, an independent blue-light receptor may be involved. Further experiments are required to distinguish between these possibilities and it is hoped that the isolation and characterisation of tropically abnormal mutant strains will be valuable in this respect.

We wish to thank the Science and Engineering Research Council for their support of this work through the award of a research grant.

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Received 13 January; accepted 5 April 1983